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Applicability of the use of visual indicators [presence of Beggiatoa and/or Opportunistic Polychaete Complexes (OPC)] to identify benthic changes due to aquaculture on various substrates

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Data from sampling conducted on aquaculture sites as well as information extracted from industry monitoring reports were used to evaluate the applicability of using *Beggiatoa* and OPC as indicators of benthic change due to aquaculture. The first set of data analyzed pertains to monitoring reports completed at cage edge; *Beggiatoa* and OPC presence were then noted with no assessment of coverage. Changes to the habitat monitoring protocols were implemented in June of 2011 with protocols changing from cage edge sampling to transect sampling (with transects located around the cage array). Data collected via transect sampling were analyzed to search for trends across sites in relation to distance to cage, and to evaluate variability between stations located in the same direction. *Beggiatoa* and OPC were not present at reference sites and were found to be associated with aquaculture activities. *Beggiatoa* and OPC presence correlate with known indicators of aquaculture activities such as flocculent presence, offgasing and sulfides (up to a certain concentration). *Beggiatoa* and OPC were found to decrease with distance from cage though exhibiting patchy distributions. Despite this patchiness, average differences in *Beggiatoa* and OPC coverage between stations along transects in the same direction were less than 10 %. When transforming data into dummy variables (absence/presence) we found the same trends suggesting that the presence/absence of these organisms could also be used as an adequate trigger to inform on waste deposition at finfish sites.

L'utilisation de la présence de *Beggiatoa* et des complexes de polychètes opportunistes (CPO) comme indicateurs de changement de l'habitat benthique attribuable à des activités piscicoles

RÉSUMÉ

Les résultats de nos études, de même que les données extraites des rapports de surveillance de l'industrie, ont été examinés pour évaluer la pertinence de l'utilisation de *Beggiatoa* et des complexes de polychètes opportunistes (CPO) comme indicateurs de changement de l'habitat benthique attribuable à des activités piscicoles. Le premier ensemble de données analysées a été extrait de rapports de surveillance menée aux quatre coins des cages; la présence de *Beggiatoa* et de CPO a alors été notée sans évaluation de la couverture spatiale (présence/absence). Des changements aux protocoles de surveillance des habitats ont été introduits en juin 2011, pour passer de l'échantillonnage au bord des cages à l'échantillonnage sur quatre transects autour de l'ensemble des cages. Les données ont été analysées pour déceler des tendances à l'échelle des sites en ce qui a trait à la distance par rapport aux cages et afin d'évaluer la différence entre stations situées dans la même direction. *Beggiatoa* et le CPO n'étaient pas présents dans les sites de référence. Selon les analyses de l'arbre décisionnel, le *Beggiatoa* et le CPO réagissent le plus aux activités d'exploitation (référence, niveau de production, mise en jachère) et, dans une moindre mesure, à la profondeur de l'eau et au type de substrat. La présence de *Beggiatoa* et du CPO est corrélée aux autres variables visuelles, comme la présence de floculant, le dégagement gazeux et le sulfure (jusqu'à une certaine concentration). Les niveaux de *Beggiatoa* et de CPO diminuent à mesure que l'on s'éloigne des cages et ont une répartition éparse. Malgré cet aspect, les différences moyennes entre les couvertures spatiales des indicateurs (*Beggiatoa* et CPO) entre les stations le long des transects situes dans la même direction étaient inférieures à 10 %. En exprimant les données sous forme de variables nominales (présence/absence), nous avons noté les mêmes tendances. La présence ou l'absence d'indicateurs pourrait être utilisée comme élément indicateur de l'enrichissement organique benthique et ceci étant donné la variabilité observée dans les valeurs de couverture spatiale.

INTRODUCTION

Over the past decade, the finfish aquaculture industry has shown significant expansion along the south coast of insular Newfoundland (NL). This area has sheltered bays, coves, fjords, and inlets, offering suitable areas for both caged and suspended gear culture (i.e. shellfish aquaculture). Approximately 90 % of finfish aquaculture sites occur in bays and coves on the south coast of the island, where water depths are greater than 30 m (DFA 2011). The siting requirements include the location of sites at depths greater than 30 m, over hard bottom substrates as these areas were expected to be highly erosional and therefore wastes were not expected to accumulate. In this region, hard and patchy substrates are predominant, and current measurements indicate that most sites have bottom current velocities les than 5.1 cm/s with no or little resuspension of organic wastes. The critical resuspension velocity as identified in a deposition model such as DEPOMOD is set at 9.5 cm/s (Chamberlain et al. 2005). These conditions (hard and patchy substrates, low bottom current) highlight the importance of monitoring waste deposition and the need for a better understanding of bioremediation processes. Conventional parameters, such as sulfides and redox potential used for soft sediment sites, have limitations in deep sites with hard or patchy substrates because of the challenges associated with obtaining sediment grabs (DFO 2005). In addition, extreme depths (> 30 m) at many culture sites on the south coast of NL have dictated the utilization of drop cameras/videos to document benthic changes instead of SCUBA surveys. This visual monitoring method is also used for regulatory purposes (DFO 2005; DFO 2013a, 2013b).

Although more than 120 biological and geochemical variables have been used to assess benthic condition near aquaculture sites (Kalantze and Karakassis 2006), none of these indicators have been fully assessed on hard and patchy substrates. Two impact indicators used on soft substrates, *Beggiatoa* sp. and opportunistic polychaete worm complexes (OPC), may have utility on hard substrates because they are conspicuous and known to occur on hard substrates near finfish sites (Emmett et al. 2005, 2007, 2008). *Beggiatoa* forms bacterial mats and may be a primary indicator as it occurs at the interface of oxic and anoxic conditions and is typically associated with elevated sulfide levels (Preisler et al. 2007). A comprehensive review of the British Columbia Ministry of Environment (BCMOE) provincial monitoring results collected at 21 active finfish farms under the Finfish Aquaculture Waste Control Regulation (FAWCR) has revealed that colourless, sulphur-oxidizing bacteria (*Beggiatoa* sp.) are present under or adjacent to finfish farms sited on hard seabeds (Emmett et al. 2005, 2007, 2008). Moreover, several authors (e.g., Weston 1990; Holmer and Kristensen 1992; Crawford et al. 2001) have found that local benthic organic enrichment under fish cages can be detected by the presence of bacterial mats. The second potential indicator, OPC, is frequently observed in areas with organic enrichment and reduced conditions at salmon farms in British Columbia (Brooks 2001). In particular, the proliferation of infaunal polychaetes within soft bottom sediments during periods of high organic input at finfish farms is well known (e.g., Brooks 2001); other epifaunal OPC may show a similar response to organic enrichment and be useful visual indicators atop hard substrates. In NL, a study of monitoring reports (at cage edge) has revealed that *Beggiatoa* and OPC are valid visual indicators of benthic change due to aquaculture and that they are present on all substrate types (Hamoutene et al. 2013). They have been recommended for regulatory purposes as their presence correlate well with known indicators of aquaculture activities such as flocculent presence, offgasing, and sulfides (Hamoutene et al. 2013). However, the question regarding their usage for the establishment of strict regulatory thresholds remains (Hamoutene 2014). These indicators are also currently used as monitoring tools by DFO in the Pacific region following the transfer of regulatory responsibility from BCMOE to DFO.

Results from our studies as well as data extracted from industry monitoring will be examined in this document to evaluate the applicability of using *Beggiatoa* and OPC as indicators of benthic change due to aquaculture. Data will be analyzed to reveal trends across sites in relation to distance to cage and evaluate variability within stations located in the same transect and/or direction. For some sites, and when production data was accessible, presence of indicators in relation to fish numbers was plotted and discussed.

THE USE OF DATA FROM MONITORING REPORTS (CAGE EDGE) TO VALIDATE THE APPLICABILITY OF VISUAL INDICATORS (HAMOUTENE ET AL. 2013)

MATERIAL AND METHODS/STATISTICAL APPROACH

The baseline survey was designed to enable both the proponents and the regulatory authorities to make informed decisions regarding site placement and site operation by providing knowledge of the specific seafloor composition prior to the implementation of aquaculture operations. In addition to the baseline information, the current monitoring program requires that video and grab samples be collected not more than two weeks before or two weeks after initiation of a fallow period (referred to as Part 1), and four to eight weeks before the end of a fallow period (referred to as Part 2). Monitoring is conducted by consultants and reports are sent to DFO's Habitat Protection Division (HPD) as part of the regulatory process.

The data used for this study were collected at cage edge, therefore reflecting a near worst case scenario situation. Video records of the seafloor are collected at the four cardinal points of each of the cages (four camera drops) as well as a panoramic continuous footage for at least one minute; each of these points represents a 'station'. When access to the four cardinal points at cage edge is difficult, consultants cover only two points at the cage limit. When sediment presence was indicated by video, triplicate grab samples were collected at each cage location, and redox and sulfide measured and recorded on each sample (DFO 2005). It is important to note that in June 2011, HPD revised this protocol so that sampling occurs along transects rather than just at cage edge (Figure 1). We analysed 26 Baseline reports, 14 Part 1 monitoring reports (post-harvest), and 18 Part 2 monitoring reports (post-fallow). Contrary to expectation, Part 2 reports were sometimes completed before or after more than 12 months post-harvest; fallow periods varied from five to 31 months. We only had corresponding Baseline and Part 1 reports from 10 sites, and corresponding Part 1 and Part 2 reports from eight sites. There was an average of 56.5 ± 30.4 stations (mean ± standard deviation) per site with video recordings in baseline reports used herein, as well as 53.5 ± 26.8 and 47.9 ± 31.7 stations for Part 1 and Part 2 reports, respectively. The data in the video description tables (as found in baseline and monitoring reports) were summarized as described below:

- Flora and fauna (#): mean number of species/taxonomic groups per station (in accordance to the level of detail provided in the table), as no abundances were recorded by the consultants;
- Flocculent matter (%): data was summarized as the percentage of stations with flocculent matter presence (number of stations with flocculent x 100 / total number of stations);
- *Beggiatoa* (%): data was summarized as a percentage of stations with *Beggiatoa* presence (number of stations with *Beggiatoa* x 100 / total number of stations);
- OPC (%): data was summarized as a percentage of stations with OPC presence (number of stations with OPC x 100 / total number of stations);

- Offgasing (%): data was summarized as a percentage of stations with noted offgasing (number of stations with offgasing x 100 / total number of stations); and
- Substrate type: percentage of presence of the following classes of substrate was determined: bedrock, coarse (boulder, rubble), medium (cobble, gravel), and fine (sand, mud), as described in Wentworth (1922). For every substrate class, percentage was calculated as follows: number of stations with substrate class x 100 / total number of stations.

The data described above were analyzed using:

- Decision tree analyses (DTA): DTA was used to explore trends in the parameters measured (dependent variables) in relation to site conditions (predictors). DTA allows the formulation of relationships between one response (i.e., dependent) variable and several predictor (i.e., independent) variables by dividing a data set recursively into smaller, increasingly statistically homogeneous portions. Exploratory analysis should be applied with no limiting assumptions about data distributions and independence of predictor variables (Breiman et al. 1984). The final result constitutes a division of the original data set into mutually exclusive and exhaustive sub-sets (e.g., Breiman et al. 1984; Quinlan et al. 1987; Biggs et al. 1991; Safavian and Landgrebe 1991; Hamoutene et al. 2008; Hamoutene et al. 2009). DTA was carried out using the procedure described by Breiman et al. (1984). At every level of the tree, stepwise splitting was performed by examining each of the predictor variables in turn, and selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The splitting criterion was expressed as a proportional reduction in error (PRE), with a minimum PRE of 0.05 required for a split to result for any given predictor/variable. The PRE constitutes the proportion of variance explained, whereby PRE is evaluated for each node as well as for the entire tree model (Breiman et al. 1984). The procedure supports both continuous and categorical variables. The risk of over fitting was controlled by specifying a minimum number of cases, or stop size, for the creation of new nodes (Puestow et al. 2001). That is, if a given node contained fewer observations than the specified stop size, it was not further partitioned. A stop size of five was selected for all tree models. Dependent variables (i.e., the indicators) that may change with potential aquaculture impact include: flora and fauna, flocculent matter, redox potential, sulfides, *Beggiatoa*, OPC, and offgasing. Categorical predictor variables used in the analyses include 'report type': Baseline, Part 1, and Part 2. Continuous predictors are: 'depth' and substrate types (4 predictors) as divided in % 'bedrock', % 'coarse' , % 'medium', % 'fine'.
- One way repeated measures ANOVA and Pearson Product Moment correlation: Comparisons between the parameters measured/assessed through video analyses and grab sampling when applicable (fauna, flora, *Beggiatoa*, OPC, redox, sulfides, flocculent, offgasing) were completed between Baseline and Part 1 reports (same sites), as well as between Part 1 and Part 2 reports, using repeated measures one-way ANOVAs. Correlations between parameters were also explored using the Pearson Product Moment correlation.

RESULTS AND DISCUSSION

In this study, we assumed that data from sites with different production levels and fallow period lengths (not all Part 2 reports were completed after one year of fallow as required) were "equivalent". Despite differences in conditions (depth, substrate, amount of production, etc.) and sampling dates, sites were grouped into three categories (baseline, Part 1 and Part 2) in order to identify dominant trends, independent of site-specific conditions, and to ensure that indicators

such as *Beggiatoa* and OPC presence were associated with aquaculture operations throughout the year.

The decision trees demonstrated that the first and most influential predictor for *Beggiatoa*, OPC, flocculent matter, offgasing presence, as well as redox and sulfides was 'type of reports' and that 'depth' and/or 'substrate type' had little or no influence on the results (Table 1). *Beggiatoa* and offgasing were the only indicators that seemed "sensitive" enough to allow the differentiation between the three scenarios: baseline, end of production (Part 1), and end of fallow (Part 2). OPC presence was influenced by depth (second split). However, when comparing the same sites at the end of production and after fallow using one-way ANOVAs (Table 2), OPC presence was higher in Part 1 reports, with a significant decrease after fallow, showing a direct link with aquaculture operations.

Table 1. Main predictors and PRE values for decision trees generated for: fauna, flora, Beggiatoa, OPC, redox, sulfides, flocculent, and offgasing. Predictors include: substrate, depth, and type of reports.

Parameter	First Predictor and data subdivision	Second predictors and data subdivision	PRE
Fauna (n=57)	Substrate	Substrate	50.8 %
Flora (n=57)	Type of reports (Baseline < Part 1 and 2)	Substrate	34.4 %
Beggiatoa (n=57)	Type of reports (Baseline < Part 1 and 2)	Type of reports (Part 2 < Part 1)	75.4 %
OPC (n=57)	Type of reports (Baseline < Part 1 and 2)	Depth	69.3 %
Redox (n=32)	Type of reports (Baseline > Part 1 and 2)	Substrate	40.8 %
Sulfides (n=32)	Type of reports (Part 1 > Baseline and Part 2)	Substrate	74.0 %
Flocculent (n=57)	Type of reports (Baseline < Part 1 and 2)	Substrate	87.7 %
Offgasing (n=56)	Type of reports (Part 1 > Baseline and Part 2)	Type of reports (Baseline < Part 2) and Substrate	58.8 %

Table 2. Pairwise comparisons between parameters (fauna, flora, Beggiatoa, OPC, redox, sulfides, flocculent, and offgasing) under different site conditions (Baseline, Part 1, Part 2). P-values are the result of repeated measures one-way ANOVAs; statistically significant (P < 0.05) values are in bold.

Parameter	Baseline-Part 1 comparison (n=10 sites)	Part 1- Part 2 comparison (n=8 sites)
Fauna	P = 0.232, no differences	P = 0.077, no differences
Flora	**P = 0.007, Baseline < Part 1**	P = 0.576, no differences
Beggiatoa	**P < 0.001, Baseline < Part 1**	**P = 0.004, Part 1 > Part 2**
OPC	**P < 0.001, Baseline < Part 1**	**P = 0.008, Part 1 > Part 2**
Redox	**P = 0.012, Baseline > Part 1**	Not enough data points
Sulfides	**P = 0.016, Baseline < Part 1**	Not enough data points
Flocculent	**P < 0.001, Baseline < Part 1**	P = 0.084, no differences
Offgasing	**P = 0.022, Baseline < Part 1**	P = 0.399, no differences

Significant positive correlations were found between flocculent presence and offgasing, as well as *Beggiatoa*, OPC, and sulfides (Table 3). No significant correlations were found between any of these indicators and redox potential values. In a report on the sediment physicochemical characteristics at seven salmon farms, Brooks (2001) found that sediment redox potential and free sulfides were negatively correlated, with Pearson correlation coefficients between -0.87 and -0.75. Similar results were also reported by Wildish et al. (1999). In this report, the absence of

significant correlation with redox potential might be due to the low numbers of observations (no grabs), as well as the inherent variability in measurements of sediment redox potential (e.g., Hargrave et al. 1993; Wildish et al. 1999). Monitoring and baseline reports revealed also that *Beggiatoa* and offgasing were absent in reference sites, though OPC was present (albeit rarely) on the benthos in areas where there was no aquaculture activity. After considering average sulfide values associated with *Beggiatoa* and OPC presence on sites (n=24), the mean sulfide concentration was 2894.7 ± 1813.8 µM (749 to 7016 µM) corresponding to oxic B to anoxic conditions as per Hargrave et al. (2008). Average redox value was -258.4 ± 159.4 mV (-407 to 412 mV) when *Beggiatoa* and OPC were found on sites (n=8). More detailed analyses (at the station level) are necessary to characterize the sulfide and redox thresholds corresponding to *Beggiatoa* and OPC presence.

Table 3. Correlation coefficients between parameters as explored using Pearson Product Moment correlation (after Bonferroni adjustment, significance is at P < 0.002). Significant correlations are in bold.

Correlation	Flora	Flocculent	Offgasing	Redox	Sulfides	*Beggiatoa*	OPC
Fauna	0.179	-0.073	-0.077	-0.031	-0.322	-0.016	0.047
Flora	*	0.219	0.102	-0.170	0.079	0.389	-0.005
Flocculent	*	*	**0.679**	- 0.462	**0.832**	**0.894**	**0.740**
Offgasing	*	*	*	-0.481	**0.625**	**0.538**	**0.416**
Redox	*	*	*	*	- 0.436	-0.430	-0.188
Sulfides	*	*	*	*	*	**0.747**	**0.632**
Beggiatoa	*	*	*	*	*	*	**0.606**

No differences were found between reports (Table 2) with respect to mean numbers of taxonomic groups (i.e.: an evaluation of richness), and a strong influence of substrate was found after the application of DTA. However, the absence of abundance/count data in the video tables precluded an accurate assessment of epibiotic diversity. Surprisingly, values for flora in Baseline reports were lower than in Part 1 and Part 2 (Table 2). This might be due to the fact, as cited above, that abundance was not evaluated, and that presence/coverage of many algae would be recorded the same as a single individual. Considering that video sampling is the primary tool used for monitoring in the NL region, it would be useful to obtain counts/abundance of fauna or flora identified in videos to ensure a better characterisation of the benthic populations (this was completed in the protocols used after this).

Baseline values for redox potential and sulfides indicate that some sites (50 % of sites investigated in this study) would already be considered post-oxic or slightly hypoxic, as defined by Hargrave et al. (2008), even before the commencement of aquaculture activity. Sulfides at reference locations away from fish cages were < 300 µM, while concentrations at farm sites varied from 150 to 5000 µM S^{2-} (Hargrave et al. 2008). The measurements collected in sites at harvest (Part 1) correspond to the values described by Hargrave et al. (2008) at production sites, though it is worth noting that Part 2 reports revealed persistent hypoxic conditions (median of 1820.9 µM) even after fallow, with OPC and *Beggiatoa* still present on most sites. Sediment chemical remediation following removal of salmon from culture cages has been defined as the return of organic and redox potentials to reference levels associated with a reduction of sulfides to values less than 960 µM (Brooks and Mahnken 2003). Our data suggest that, despite a fallow period, sites still show hypoxic conditions and the presence of indicators of organic enrichment such as *Beggiatoa*, OPC, flocculent matter, and offgasing. Variation in the duration of the fallow periods precluded any conclusions on trends.

Beggiatoa and OPC were not found in reference sites and are visual indicators of aquaculture effect on the benthos independently of substrate type. They can be used for regulatory

purposes and correlate well with known indicators of aquaculture activities such as flocculent presence, offgasing and sulfides. Our results suggest that benthic hypoxic conditions may exist in some sites prior to aquaculture activities. This report highlights the importance of collecting counts/abundance of fauna and flora in order to better evaluate epibiotic richness.

ASSESSMENT OF THE VARIABILITY OF VISUAL INDICATOR COVERAGE WITH DISTANCE FROM CAGE AND WITHIN DUPLICATE TRANSECTS

As stated previously, improvements and changes to the habitat monitoring protocols were implemented in June 2011. In particular, the protocol was changed from cage edge sampling to transect sampling (3 transects around the cage array (Figure 1). A better quantification of OPC and *Beggiatoa* presence is completed through the evaluation of percent coverage using a frame of 50 cm x 50 cm as a reference. Counts/abundances of fauna and flora are recorded in order to better evaluate epibiotic richness. All the results discussed below pertain to a transect approach in site monitoring.

MATERIALS AND METHODS

We processed visual sampling data extracted from habitat monitoring reports (9 Part 1 reports as well as video data we collected at 13 aquaculture sites. General characteristics of the sites are provided in Table 1, 2, and 3.

Monitoring reports

Underwater video is collected along transects extending from the cage edge at each corner of the cage array. Transect sampling follows a tiered approach with underwater video completed at 10 m intervals starting at the cage edge (0 m) and continuing for a maximum of 50 m from the edge (Figure 1). Sampling is often discontinued after 30 m from cage edge if no changes are visually detected in the benthic conditions. Sampling is generally not completed at stations >100 m in depth. Video footage is recorded using a custom built (Falkjar) underwater video camera powered by NTSC operational system mounted to an aluminum frame (50 cm x 50 cm) on a 90° angle pointing vertically at the seafloor. Live video footage is recorded on a JVC camcorder and GPS coordinate positions are overlain onto the video through use of a Sea-Trak Video Overlay system by SeaViewer. The video observation tables listed in the monitoring reports include the following information: GPS coordinates, depth (m), substrate type (categorized based on the Wentworth scale), a list of species/groups of fauna and flora present. Still images of the video footage are captured and Image J software is used to determine percent coverage of substrate type, *Beggiatoa*, and OPC. Flora and fauna groups are counted or described as: prevalent, some, few, or rare. Flora (coralline algae and seaweed) and fauna: echinoderms (feather star, brittle star, sea urchin, sea star); molluscs; sponges; soft corals; and anemones are counted as described in the list of NL species documented so far (DFO 2013a). Due to some differences in data reporting, the classification for the different variables was standardized to ensure the same level of detail is provided. Specifically, percentages for flocculent material were sometimes not reported; in these instances, values were assigned depending on the descriptors available in the table (100 % when flocculent was the only substrate type reported; 50 % if another substrate was cited; and 33 % or less if more than one substrate was observed). For this study, 9 Part 1 reports were analysed. Time of year when sampling was completed was variable across sites.

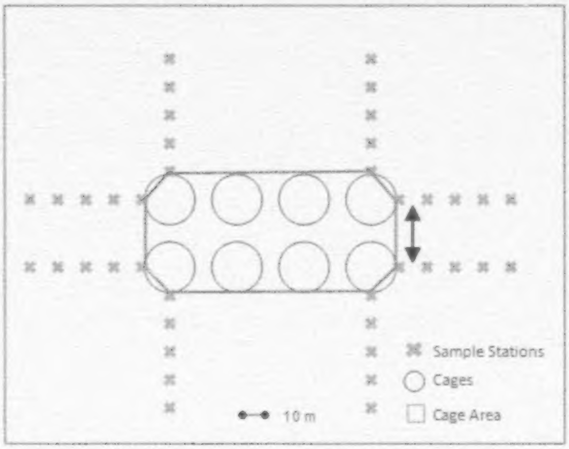

Figure 1 – Sampling design used for monitoring reports: Part 1 (after two years production). Variability evaluation (as described below) using percent coverage values of all stations in duplicate transects.

Sampling at finfish sites

Video collection protocols used by our team are similar to those used today by industry for monitoring purposes (DFO 2013b). Two different sampling campaigns were completed. The first sampling (July-August 2010) was extensive and allowed a comprehensive coverage of lease areas with a higher surface area covered at every camera drop. The second one (July to September 2011 and June to August 2012) mimicked the point source approach used for monitoring protocols with the same surface area covered at every camera drop (50 cm x 50 cm) (DFO 2013b). The collected video data was summarized in table format with the following information: GPS coordinates, depth (m), substrate type, and counts of species/groups of fauna and flora present as described above. Substrate was reported as percentage of each type observed. Similarly to Beggiatoa, OPC, and flocculent matter, flora, such as coralline algae and seaweed, were recorded as a percentage of bottom cover by defining regions of interest by hand using Image J (DFO 2013b). No coverage of flocculent matter was evaluated for the first sampling completed in 2010.

July-August 2010

Underwater video was collected in July and August 2010 on 5 finfish (Atlantic salmon, *Salmo salar*) aquaculture sites. Video sampling was performed along 6 transect lines at each site: three parallel to, and three perpendicular to the coastline (Figure 2). Along each transect, sampling stations were spaced by 50 m, and the number of sampling stations per site varied according to the spatial extent of the lease. Each video recording lasted at least 1 minute. The benthic area covered during each video recording varied between stations due to movement of both the camera and boat (drift) and represented on average 3.02 ± 0.31 m2 (coefficient of variation of 10.26 %). The surface covered was considered as equivalent allowing us to group all observations for data analyses. No video collection took place in depths in excess of 100 m.

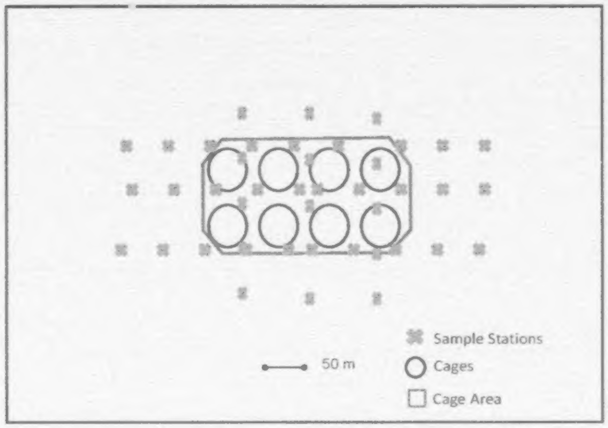

Figure 2 – Sampling design for data collected at finfish aquaculture sites in July-August 2010 (Example for one site, not all transects had the exact same pattern depending on lease areas).

July to September 2011 and June to August 2012

Video data were collected at 8 finfish (Atlantic salmon) aquaculture sites using the new protocols established for industry monitoring reports; however, the transect lines were longer (160 m with stations 20 m apart) and fewer (3) on a per site basis (Figure 3). A reference site was also sampled with no cage presence. Three transects were completed per site with two oriented parallel and one oriented perpendicular to the cage array (Figure 3). Stations were typically not sampled if water was too deep (>100 m), or if located too close to shore. The collected video data was collected similarly to what is described above.

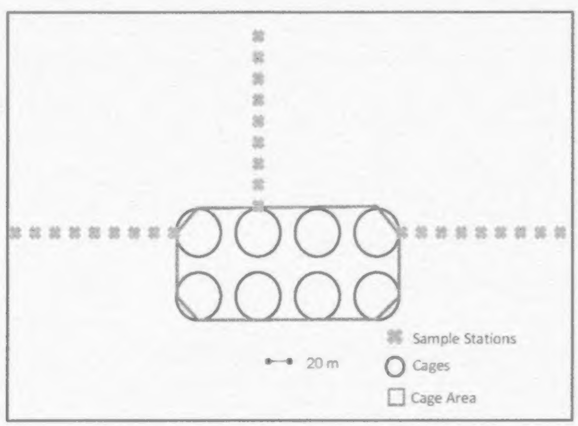

Figure 3 – Sampling design for data collected at finfish aquaculture sites from July to September 2011 and from June to August 2012.

Water column speed and direction were collected and analyzed at some sites using moored ADCP (Acoustic Doppler Current Profilers) in model A2 SUBS system and data are provided for information.

Statistical analyses

In addition to stating general site characteristics, percentages of stations with indicators on sites are calculated (Table 1, 2, and 3). Mean percent coverage of all stations (as determined by

ImageJ) of *Beggiatoa*, OPC, and flocculent material (all values) is plotted to visualize distribution with distance from cages for the 2011 and 2012 data set (Figure 4). Correlations between percent coverage (*Beggiatoa*, OPC and flocculent) and distance from cage are explored using Pearson Product Moment and Spearman Rank (for data not normally distributed). Data were arcsin square root transformed prior to statistical analyses, but this transformation showed no effect on results. Arcsin square root transformation is necessary when a sizeable number of the observed proportions are either relatively small ($P < 0.2$) or large ($0.8 < P < 1$); if most of the computed proportions lie between 0.2 and 0.7, it should have little impact on the results (Snedecor and Cochran 1980). Percent coverage data were also transformed in dummy variables (0: absence, 1: presence) to explore data correlation with distance from cage (Point-biserial correlation). This was completed for sites sampled in 2010, 2011 and 2012 as well as Part 1 reports. For sampling completed in 2010 some stations were located below cages; distance to cage was considered as zero for all these stations.

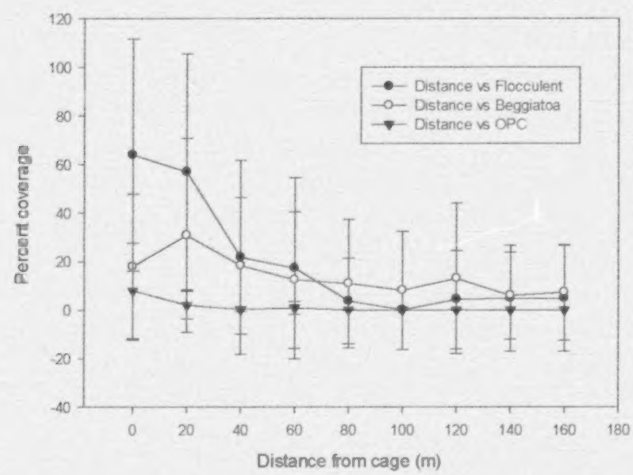

Figure 4 – Mean percent cover of Beggiatoa, OPC, and flocculent material with distance from cages (m) using data extracted from eight Newfoundland aquaculture sites (after one year production in 2011 and 2012). N varies from 21 to 27 stations for every distance from cage.

Data from monitoring reports (Part 1) were used to assess differences between duplicate transects (same distance from cage and same direction). Transects in the sites considered for this study were separated by an average distance of 160 m (40 to 450 m). *Beggiatoa* and OPC percent coverage differences are calculated by subtracting values from stations at equal distance from cages and belonging to transects in the same direction (as per Figure 1). Means and standard deviations of these differences are calculated for both *Beggiatoa* and OPC coverage.

RESULTS

Site characteristics

An overview of the general characteristics of sites (sampled sites, and Part 1 reports) is provided in Tables 4, 5, and 6. Percentages of stations with indicators were calculated in order to give an overview of *Beggiatoa*, OPC and flocculent presence on sites. Production length and fallow periods in months are also provided in the tables. When production values were available

to us, numbers of fish stocked on sites are added to the tables. All sites have a mean depth above 30 m with a variety of dominant substrates across sites.

Table 4- General characteristics of the sites sampled in 2010.

Site #	N	Depth range (m)	% St. with *Beggiatoa*	% St. with OPC	% St. with Floc.	Dominant substrates	# of cages	# Fish Stocked	Prod. Time in months
1	32	15-88	3.0	6.1	7.40	Fine, Bedrock	16	881,822	4
2	32	17-54	2.0	0.0	29.63	Fine, Coarse	10	1,000,000	4
3	42	10-80	0.0	14.3	52.00	Fine, Medium	6	387,000	16
4	48	7-67	14.6	8.3	45.00	Fine	6	607,000	13
5	27	6-93	29.6	18.5	17.65	Fine, Medium	9	269,000	25

St.: stations, Floc.: flocculent, Prod.: Production, N=number of stations per site.

Table 5- General characteristics of the sites sampled in 2011 and 2012 (one year production).

Site #	N	Depth range (m)	%St. with *Beggiatoa*	% St. with OPC	% St. with Floc.	Dominant substrates	# of cages	# Fish Stocked	Prod. Time in months
6	27	40-49	37.04	3.70	7.40	Sand, Cobble	10	919,816	12
7	27	24-60	48.15	22.22	29.63	Sand, Cobble	16	498,964	12
8	25	77-117	44.00	20.00	52.00	Sand	10	971,169	15
9	20	46-138	35.00	0.00	45.00	Bedrock	6	630,983	12
10	17	62-141	76.47	5.88	17.65	Boulder	6	793,208	14
11	20	46-138	30.00	30.00	25.00	Sand, Cobble	12	403,000	13
12	44	21-72	9.09	0.00	6.82	Sand, Cobble	16	530,000	12
13	32	22-55	9.37	0.00	15.62	Silt/Mud	11	383,000	12

St.: stations, Floc.: flocculent, Prod.: Production, N=number of stations per site.

Current velocities were available only for sites #6 and #10 with a mean (depth-averaged) current velocity of 6.33 cm/s and 6.02 cm/s, respectively. Bottom velocities (average of 1 m at the bottom) are equal to 5.1 cm/s for site #6 and 2.9 cm/s for site #10.

Table 6- General characteristics of the sites described in the Part 1 monitoring reports.

Site #	N	Depth range (m)	% St. with *Beggiatoa*	% St. with OPC	% St. with Floc.	Dominant substrates	# of cages	# Fish Stocked	Prod. Time in months
14	28	35-65	64.28	0.00	7.14	Silt/Mud, Cobble	16	521,000	23
15	33	43-85	18.18	0.00	0.00	Silt/Mud	10	300,000	24
16	35	18-60	31.43	22.86	22.86	Silt/Mud, Cobble	6	495,720	26
17	32	24-61	9.37	6.25	6.25	Silt/Mud, Cobble	12	317,000	23
18*	21	30-54	23.81	0.00	0.00	Silt/Mud, Cobble	7	n/a	31
19	36	16-48	47.22	2.78	2.78	Silt/Mud, Cobble	6	n/a	23
20	38	40-100	26.32	55.26	55.26	Silt/Mud, Bedrock	6	905,092	24
21	34	28-45	29.41	0.00	0.00	Silt/Mud	14	520,000	25
22	50	12-63	28.00	30.00	30.00	Silt/Mud, Gravel	16	310,000	28

*cod site, St.: stations, Floc.: flocculent. N=number of stations per site.

Water current data was only collected in the vicinity of site #16, where a mean value of 2.98 cm/s and a bottom current of 2.1 cm/s were obtained. The length of production for sites described in Table 2 was around 2 years though some sites had longer production cycles as described in monitoring reports.

Percent coverage of visual indicators with distance from cage

Mean percent coverage at stations located at the same distance from cages was calculated and plotted for each visual indicator. Data for the 8 sampled sites in 2011-12 are presented in Figure 4. All three indicators declined in percent cover with distance from the cage. *Beggiatoa* was observed as far as 160 m from the cages while OPC was generally not observed beyond 80 m. Flocculent material occurred within 100 m of the cages. Standard deviations (SD) of means (across sites and transects) represent variability across sites and stations located in different transects/directions, but at a similar distance from cages. For all data (2010, 2011-12 sampling and Part 1 reports) coefficients of variation for all stations across sites and transects (SD/mean x 100) increased with distance from cage for all three visual parameters and all data (from 70 % to 450 %). Overall, *Beggiatoa*, OPC and flocculent are negatively correlated with distance from cage though correlation coefficients are low and comprised between -0.189 and - 0.528 (Tables 7, 8, 9). These values are equivalent after transformation of data in dummy variables (values between brackets). Statistically significant correlations are observed between *Beggiatoa* presence, OPC and flocculent (all pairwise correlations except that OPC and *Beggiatoa* are not always correlated, especially when considering percent coverage).

Table 7- Correlation coefficients and associated probabilities (Spearman rank order) between distance and percent coverage (first line) or presence/absence (values between brackets) of Beggiatoa, OPC, and flocculent. For Part 1 monitoring reports, n = 307 stations.

Correlation	Floc. Part 1	Beg. Part 1	OPC Part 1
Distance	-0.095, P=0.097 (-0.104), P=0.070	**-0.189, P<0.001 (-0.187), P<0.001**	0.031, P=0.589 (0.039), P=0.496
Floc. Part 1	*	**0.355, P<0.001 (0.324), P<0.001**	**0.587, P<0.001 (0.575), P<0.001**
Beg. Part 1	*	*	0.074, P=0.198 (0.088), P=0.123

Beg=*Beggiatoa*; OPC=opportunistic polychaete complexes; Floc=flocculent material. Statistically significant correlations (P < 0.001 or P<0.05) in bold.

Table 8- Correlation coefficients and associated probabilities (Spearman rank order) between distance and percent coverage (first line) or presence/absence (values between brackets) of Beggiatoa, and OPC. For sampled sites in 2010, n = 182 stations.

Correlation	Beg. Sampled 10	OPC Sampled 10
Distance	**-0.254, P<0.001 (-0.254), P<0.001**	**-0.290, P<0.001 (-0.292), P<0.001**
Beg. Sampled 10	*	-0.020, P=0.790 **(0.210), P<0.05**

Beg=*Beggiatoa*; OPC=opportunistic polychaete complexes. Sampled 10=sampled in 2010. Statistically significant correlations (P < 0.001 or P<0.05) are in bold

Table 9- Correlation coefficients and associated probabilities (Spearman rank order) between distance and percent coverage (first line) or presence/absence (values between brackets) of Beggiatoa, OPC, and flocculent. For sampled sites in 2011-12, n = 235 stations.

Correlation	Floc. Sampled 11-12	Beg. Sampled 11-12	OPC Sampled 11-12
Distance	**-0.528, P<0.001 (-0.523), P<0.001**	**-0.331, P<0.001 (-0.349), P<0.001**	**-0.347, P<0.001 (-0.346), P<0.001**
Floc. Sampled 11-12	*	**0.531, P<0.001 (0.530), P<0.001**	**0.571, P<0.001 (0.587), P<0.001**
Beg. Sampled 11-12	*	*	**0.395, P<0.001 (0.399), P<0.001**

Beg=*Beggiatoa*; OPC=opportunistic polychaete complexes; Floc.=flocculent material. Sampled 11-12: sampled in 2011 and 2012. Statistically significant correlations (P < 0.001 or P<0.05) are in bold

Percent coverage of visual indicators within same direction transects (Part 1 reports)

In order to better assess variability, we calculated differences in percent coverage in *Beggiatoa* and OPC between stations located at the same distances from cage and belonging to same directional transects (as per Figure 1). A total of 130 stations were considered (60 transects in total). A mean difference of 5.7 ± 8.9 % was found for *Beggiatoa* coverage and 1.5 ± 9.8 % for OPC percentages.

Presence of visual indicators in relation to fish production levels

Correlations were evaluated between production levels (fish numbers) and percentages of stations with *Beggiatoa* or OPC on the corresponding sites. Sites were separated according to length of production and sampling procedures. Ten sites at the end of their first year of production were sampled by our team (2010, 2011 and 2012) and 7 sites as per Part 1 reports. Production and % stations with *Beggiatoa* had a correlation coefficient (R) of 0.600 (P=0.067) in sites after one year of production; after a 2 year production cycle, R = 0.259 and P = 0.574. The percentages of stations containing OPC and fish numbers had R = -0.093 and P = 0.826 after one year; and R = 0.655 and P = 0.110 at the end of two years.

DISCUSSION

The site conditions considered differ in terms of production levels, depth, substrate, water currents, and operator. Data related to sites are not compared statistically but provide a general idea of the variability and distribution of visual indicators. Results of previous studies (Henderson and Ross 1995) suggest the need for a site-specific approach, though regulatory frameworks require a general method. Our aim is to use data from different sites to ensure observations and trends are not site specific and that general recommendations can be made.

Compared with ROVs, drop-down cameras are operated at a lesser financial cost and the drop-and-drift video approach works well in achieving a high spatial accuracy (Wilding et al. 2012). The short-duration camera drops allow true spatially independent sampling which overcomes some of the statistical problems associated with contiguous transect surveys (Malatesta et al. 1992). Nonetheless, the patchy distribution and relatively low density of the megabenthos means that, relative to benthic grabs, large areas of seabed need to be surveyed in order to make a statistically (representative) number of observations (Wilding et al. 2012). After video assessment of environmental impacts of salmon farms, Crawford et al. (2001) showed that video data suggest an improvement in environmental conditions, while benthos samples (grabs) implied a continuation of degraded conditions. This difference might be related to the fact that the video data represent the sediment surface, while the benthic community samples reflect conditions within the sediment (Crawford et al. 2001). Recovery after an organic enrichment event has been shown to occur more rapidly at the surface than within the sediments (Pearson and Rosenberg 1978). Moreover, Crawford et al. (2001) show that video data can be used to separate heavily affected transects from unaffected ones, but cannot readily discriminate between intermediate and unaffected transects. In our assessment, we noted a couple of limitations in data collection when evaluating *Beggiatoa* and OPC:

- *Beggiatoa* (white mats) will often cover/colonize all surfaces not allowing us to properly identify the substrates below and in some cases to observe OPC that might be present deeper in the sediment/flocculent (as observed in a few grabs) therefore underestimating the presence of OPC.
- We were not able to take into account the thickness of bacterial mats in video data.

Percent coverage of all indicators decreased with distance from cage as a result of aquaculture waste dispersion. Correlations with distance are significant though coefficients are low. Our data highlights some of the spatial patchiness of *Beggiatoa* and OPC coverage on aquaculture sites. This patchiness was also observed in sediment chemistry data obtained after grab samples in sites in British Columbia (Brooks and Mahnken 2003). A study using Bayesian networks to examine fish farm benthic impacts poorly predicted the occurrence of *Beggiatoa* sp. mats based on distance from cage (Giles, 2008). This patchiness might be a reflection of the protocols used for monitoring sites as well (transects approach, surface area limitations). In most of our sampling and the monitoring protocols' design, the spatial resolution (50 cm x 50 cm) of the sample point is likely too coarse to accurately interpolate the percent coverage data across the entire site. With a significantly increased sampling effort and considering that the percentage cover of indicators is a spatially auto-correlated variable (as suggested by the correlations) it may be possible in the future to interpolate/model total coverage throughout the sampling area. The variability of the coverage increased with distance from cage. The variability itself (CV from 70 % to 450 % with distance) captures differences between sites and transects and might not have a real informative value (this highlights the difficulty in choosing a compliance threshold). On the other hand, the increasing trend in variability with distance is to be noted as well as expected considering the fact that dispersion of aquaculture wastes around cages is not homogeneous. When considering stations located on transects in the same direction (assumption is made that same direction equals similar water current conditions), and even if transects were more than 100 m apart, differences in percent coverage of *Beggiatoa* and OPC did not exceed 6 % (up to 15 % when considering SD).

Correlations with distance show similar coefficients when considering presence/absence of indicators. It remains challenging to link a particular percentage of coverage with a known effect on the benthos especially within the limitations imposed by sampling protocols. Moreover, a basic problem with the use of standards of fixed numerical criteria is that they do not allow for natural variability in space and time if this variability is not well documented (Levings et al. 2002). Presence/absence of indicators could be also used as an adequate trigger to inform on waste deposition at finfish sites.

Indicator presence (% of stations) was not significantly correlated with fish numbers on aquaculture sites at a probability value of 0.05. A positive trend significant at $P < 0.10$ emerged after one year of production for *Beggiatoa*. These results are a reflection of the fact that stocking densities (i.e. feeding strategies, husbandry) can be very different from site to site depending on the aquaculture operator even with equivalent fish numbers. Chang and Page (2011) found some significant positive correlations between the number of salmon on site and the sediment sulfide concentration, and between the biomass of salmon on site and the sediment sulfide concentration; however, there was considerable variation in these relationships. The correlations between sediment sulfide concentration and the numbers/ biomass of salmon appeared to be strongest when the numbers/ biomass were at low to intermediate levels; the relationships appeared to disappear at higher numbers/ biomass, and sediment sulfide concentrations were quite low at the farms with the highest numbers/biomass (Chang and Page 2011). Similar findings were reported at salmon farms in British Columbia (Brooks 2001; Brooks and Mahnken 2003), where it was found that sediment sulfide concentrations increased during early stages of farm production, when biomass and feeding rates were low, but that benthic effects did not increase linearly with increasing production. Moreover, other factors such as bathymetric profiles and water currents have to be considered to ensure a full understanding of deposition in relation to production.

GENERAL CONCLUSIONS

Beggiatoa and OPC were not present in reference sites and found to be linked with aquaculture activities on all substrate types. *Beggiatoa* and OPC presence is correlated with known indicators of aquaculture activities such as flocculent presence, offgasing and sulfides at the site level. Indicators were found to decrease with distance from cage though exhibiting patchy distributions. When transforming data in dummy variables (absence/ presence) we found the same trends suggesting that presence/absence of indicators could also be used as an adequate trigger to inform of waste deposition at finfish sites. Our results also suggest that benthic hypoxic conditions may exist in some sites (baseline reports) prior to aquaculture site set-up.

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